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Proc. R. Soc. Lond. B 1907 **79**, 130-154
doi: 10.1098/rspb.1907.0012

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The Chemistry of Globulin.

By William Sutherland, M.A. Melbourne.

(Communicated by Dr. C. J. Martin, F.R.S. Received July 26,—Read December 6, 1906.)

The object of the present paper is first to establish simple formulæ for the more important of the experimental results obtained by Hardy* and Mellanby,† then to interpret these in their bearing upon the chemistry of globulin in connection with a theory of colloids, and finally to find the molecular mass (weight) of globulin. The work will be taken according to the following table of contents.

1. Formula for the solution of globulin in solutions of neutral salts, and the laws associated with this formula.
2. Solution of globulin in acids and alkalies.
3. Formulæ for the precipitation of globulin by salts and by acids.
4. A theory of the colloidal state.
5. The electric conductivity of globulin solutions.
6. The molecular mass (weight) of globulin.

1. *Formula for the Solution of Globulin in Solutions of Neutral Salts, and the Laws Associated with this Formula.*

Mellanby sets forth his results in graphs, from which he makes some broad deductions, to be discussed later in this section. His most striking result, obtained also by Hardy, is that the fraction (or percentage) of a globulin suspension which is dissolved by a salt solution of given strength is nearly independent of the original strength of the suspension. In other words, the amount of globulin dissolved by a given salt solution is proportional to the amount of globulin presented for solution in the form of a suspension. This fact is of fundamental importance for the colloidal globulin of the suspension. For example, a 0·3-per-cent. NaCl solution dissolves from a 3·04-per-cent. suspension of globulin in 100 c.c. 1·12 grammes, from 1·68 per cent. 0·64, from 1·06 per cent. 0·39, and from 0·71 per cent. 0·30.

Let G be the original strength of a globulin suspension when it is instantaneously distributed through a solution of a salt of concentration c, G and c being given in grammes per cubic centimetre. Let g be the grammes of globulin per cubic centimetre dissolved from the suspension when saturation is reached, and let C be the concentration of the salt

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* 'Journal of Physiology,' 1905, vol. 33, p. 251.

† *Loc. cit.*, p. 338.

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required to just dissolve the whole of G. Denote g/G by p, and c/C by q, then Mellanby's results are expressed by the equation

$$p(1-q) = Aq(1-p), \tag{1}$$

in which A is a parameter having characteristic values for each type of neutral salt. To illustrate the applicability of this equation, the following comparison is made of the values of $p(1-q)/q(1-p)$ for the different values of c and p given by Mellanby for his first set of data for NaCl. C was not actually measured by Mellanby, but is obtainable from his first graph with only a small extrapolation as 0·0052. Hence, from Mellanby's tabulated values of c, we obtain the values of q, given in the table along with his values of p. The last row contains the ratio $p(1-q)/q(1-p)$.

Table I.

$10^4 c$	50	45	40	35	30	25
$10^3 q$	962	865	769	673	577	481
$10^3 p$	910	830	665	555	450	356
$10^2 \dfrac{p(1-q)}{q(1-p)}$	40	76	60	61	60	60

The first two values are aberrant, because $1-q$ and $1-p$ being small are powerfully affected by experimental uncertainty. If we take $p(1-q)/q(1-p) = 0{\cdot}60 = A$, and use the equation to calculate p from q, we find for p in these first two cases 0·938 and 0·794 in place of 0·91 and 0·83, the differences being within the limits of experimental error.

Mellanby shows that the graphs for NaCl, KCl, and NH_4Cl coincide when c expressed as gramme equivalents is made abscissa and p ordinate. Hence the value of A just found for NaCl, namely 0·60 holds for KCl and NH_4Cl. Mellanby generalises this result for all neutral salts composed of two monovalent ions, but in the appendix to his paper he points out that the graphs for $K\bar{A}$, KBr, and KI do not coincide with that for KCl, a result previously found by T. B. Osborne, and given also by Hardy. Mellanby finds that a single graph will suffice for Na_2SO_4, $K_2SO_4(NH_4)_2SO_4$, $MgCl_2$, $BaCl_2$, and $CaCl_2$. For this type, namely, a divalent and two monovalent ions, I find $A = 0{\cdot}613$, say 0·61, and for the type $MgSO_4$ $A = 0{\cdot}66$.

One of Mellanby's discoveries leads towards a useful generalisation in connection with A. He measures the average solvent power of any salt for globulin up to the point when the whole of a suspension is dissolved, and finds this proportional to the sum of the squares of the valencies of its ions. For example, if the solvent power of Na is 1^2 and of Cl is 1^2, that of NaCl will be 2, that of $MgCl_2$ will be 2^2+2 or 6, and that of $MgSO_4$ 2^2+2^2 or 8. It seems simpler to measure solvent power by the reciprocal of the concentration required just to dissolve the whole of a given globulin suspension. From Mellanby's graphs we find this concentration C to be 0·000094 E for salts of the type NaCl, E being the gramme equivalent. For the type $CaCl_2$ and Na_2SO_4 it is 0·000065 E and for $MgSO_4$ 0·000056 E. Hence, for 1/C for these three types, we get the values 10600/E, 15400/E, and 17850/E. If we use gramme molecular mass M instead of gramme equivalent E, these become 10600/M, 30800/M, and 35700/M, in which the numbers are as 2 : 6 : 7, corresponding to the ratios 2 : 6 : 8 found by Mellanby when using average solvent powers. We can regard this result in a different way. Let us write (1) in the form

$$p\left(1-\frac{c}{C}\right)=\frac{A}{C}c(1-p), \qquad (2)$$

and study A/C for each type, thus making the absolute concentration c prominent. The values of A/C for the three types are 6400/M, 18800/M, and 23600/M, the numerical parts of which are MA/C, and are more nearly as 2 : 6 : 8. From this point of view, we find Mellanby's law apply better to MA/C than to M/C, to an average value of which Mellanby applied it. In Section 4, I shall try to give a theoretical explanation of Mellanby's law applied to MA/C. Meanwhile, to complete the discussion of experiments bearing on A, we shall consider his results concerning the influence of temperature on the solvent power of NaCl for globulin. He comes to the conclusion that the solvent power is proportional to the electric conductivity or summed ionic velocities of the solution of NaCl at the temperatures used. But it will be better to investigate the effect of temperature on A and A/C, and then follow Mellanby in taking account of ionic velocities in this connection.

The following table contains the values of A which I have calculated from Mellanby's data* at the five temperatures, also the values of C/E derived from his graphs. The values of EA/C are also tabulated to give relative values of A/C. The column marked η gives the viscosity of water at the five temperatures, and the last column ηEA/C :—

Table II.

Temp.	10^3A.	10^6C/E.	EA/C.	$10^4\eta$.	ηEA/C.
° C.					
1·5	860	168	5120	172	88
20	600	102	5860	101	59
30	536	84	6310	80	50
40	512	75	6790	66	45
50	605	71·5	8470	55	47

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* *Loc. cit.*, p. 353.

The value of A attains a minimum between 30° and 50°, probably between 30° and 40°. It is worth noticing that this minimum comes near to the temperature of warm-blooded animals. The viscosity of water controls the velocities of an ion at different temperatures and is inversely proportional to them. So the column headed $\eta EA/C$ gives relative values of the ratio of EA/C to the velocities of Na and Cl at the five temperatures.

These show a minimum about 40° and can be expressed by

$$\eta EA/C = 45 + 0{\cdot}031\,(t-40)^2. \qquad (3)$$

This equation makes the value of A/C at different temperatures depend not only on the viscosity η, which determines the velocities of the ions, but on specific properties of the globulin, which are a function of the temperature. Certainly one would expect, *a priori*, that the profound dependence of the chemical and physical properties of proteid on temperature would appear in such an equation; one would expect the approaching coagulation at 75° to be foreshadowed. It is again striking that the temperature of minimum $\eta EA/C$ is about 40°, near that of warm-blooded animals. These experiments have yielded in equation (1) the result that the chemical equilibrium between globulin and neutral salts is of a simple type, dependent, as far as the salt is concerned, upon ionic electric charges and velocities, but also dependent upon special properties of globulin.

It has already been mentioned that $K\bar{A}$, KBr, and KI are exceptional to a certain extent and show that other properties of salts than ionic charges and velocities have an influence on their chemical equilibrium with globulin. Moreover, the solvent powers found by Hardy* for globulin from ox blood do not agree closely with those given by Mellanby for horse globulin. For instance, Hardy finds $MgSO_4$ less powerful than K_2SO_4, whereas Mellanby finds its power 8/6 of that of K_2SO_4. Hardy finds KNO_3, $NaNO_3$, and NH_4NO_3 1·5 times as powerful as KCl and NaCl. Yet Hardy's data on the whole show that valency is the chief factor controlling solvent power. He finds K and NH_4 oxalates to have the same power as $(NH_4)_2SO_4$ and an equivalent of Na citrate 3·3 times as powerful as NaCl, so the gramme molecule would be 9·9 times as powerful as that of NaCl. On Mellanby's principle it would be $(3^2+3)/2$ or six times as powerful. Further investigation must clear up these points.

2. *Solution of Globulin in Acids and Alkalies.*

Hardy's experiments show that the reactions between globulin on the one hand and acids and alkalies on the other take place under the usual chemical conditions of combination. Whereas the amount of a globulin suspension dissolved by a certain strength of NaCl is proportional to the original concentration of the suspension, the amount dissolved by HCl depends only on the total amount of acid which has access to the suspension. Hence, for acids and alkalies in place of (1), we have the relation

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* *Loc. cit.*, pp. 308 and 309.

$$g = Bc, \tag{4}$$

where B is a parameter characteristic of each acid and alkali. Mellanby established the same result and shows that B does not vary with temperature. The contrast between solution in neutral salts and in acids or alkalies is complete. Hardy proves that a given amount of globulin reacts not with equivalents, but with molecules of such acids as HCl, H_2SO_4, and H_3PO_4. Similarly he finds that the molecule $Ba(OH)_2$ has the same solvent power as KOH, NaOH, and NH_4OH. To dissolve one gramme of globulin 10×10^{-5} gramme equivalent of alkali is required, and 18×10^{-5} gramme equivalent of the strong acids. These results will be used in Sections 4 and 6. T. B. Osborne, from 1899 to 1901, obtained similar results for the vegetable globulin edestin.

3. *Formulæ for the Precipitation of Globulin by Salts and by Acids.*

The first case is that in which globulin is precipitated by excess of a solvent neutral salt. Let c be the concentration of the salt and p the fraction which the precipitated globulin is of the total originally in solution, then Mellanby's graph for $(NH_4)_2SO_4$* is given by the equation

$$p(1+p) = 28 \cdot 8\,(c - 0 \cdot 152). \tag{5}$$

The values of c given by this for the experimental values of p are compared with the experimental values of c in the following table:—

Table III.

10^3p ……………	315	447	533	695	787	858	1000
10^3c exp. ………	166	175	183	192	200	208	218
10^3c calc………	166	174	180	193	202	207	221

To interpret this equation, we start with the consideration of the value c_0 of c at which precipitation just begins. Two actions of the salt just balance here, the solvent action and the precipitant. If, then, by increasing the concentration to c, the fraction p of the globulin is precipitated, an amount of solvent power proportional to c_0p is liberated, so the solvent power, which at c_0 was proportional to c_0, at c is proportional to $(1+p)\,c_0$. This implies that the whole of the additional salt $c-c_0$ is occupied in keeping the precipitate down. This solvent power proportional to $(1+p)\,c_0$ acting on the precipitate p will give a rate of solution of the precipitate proportional to $p\,(1+p)\,c_0$, and this must be equal to the rate of precipitation when equilibrium is reached. But as $c-c_0$ is occupied in keeping the precipitate down, the simplest assumption to make is that the rate of precipitation is proportional to $c-c_0$. Hence, for equilibrium,

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* *Loc. cit.*, p. 357.

$$p\,(1+p) = \mathrm{F}\,(c-c_0), \qquad (6)$$

where F is a parameter characteristic of each salt.

This equation of equilibrium is of the same form as (5). The precipitate in this case is probably not globulin, but a compound of globulin and the salt. It begins to come down at a concentration about 40 times that which produces complete solution of the original globulin. But Mellanby's data for $MgSO_4$ cannot be expressed by an equation like (5). They could be represented by portions of two straight lines.

When a salt of a heavy metal is added to a solution of globulin in a neutral salt, the strength of the neutral salt being kept constant in all the comparisons, the fraction of the total globulin precipitated is proportional to the amount of the salt of heavy metal up to a certain point.

Thus, with globulin dissolved in 0·6 per cent. NaCl and then precipitated with $ZnSO_4$, also dissolved in 0·6 per cent. NaCl, so that the total amount of globulin in 10 c.c. was always the same, the fraction p of the globulin precipitated is given by

$$p = 2730c, \qquad (7)$$

up to $c = 0{\cdot}000125$ gramme per cubic centimetre, c being here the grammes of $ZnSO_4$ per cubic centimetre of the 10 c.c. used. From $c = 0{\cdot}000125$ to $c = 0{\cdot}000225$ the formula is

$$p-0{\cdot}35 = 1030\,(c-0{\cdot}000125). \qquad (8)$$

Mellanby converts these two lines in his graph into a single curve, but with some violence to the experimental data. For a solution of globulin containing 0·0027 gramme NaCl per cubic centimetre and $ZnSO_4$ as precipitant, $p = 236\,c$ up to $c = 0{\cdot}001$, while from $c = 0{\cdot}001$ to $c = 0{\cdot}0035$ we can write $p - 0{\cdot}15 = 82\,(c - 0{\cdot}001)$.

For $CuSO_4$ the corresponding equations are $p = 3660\,c$ up to $c = 0{\cdot}00015$, and $p - 0{\cdot}55 = 2000\,(c - 0{\cdot}00015)$ up to $c = 0{\cdot}00025$.

In this connection it is interesting to review the considerable amount of analytical work done on the albuminates of the heavy metals.* Harnack found, in 1881, that copper albuminate precipitated from excess of albumin contains 1·35 per cent. of Cu, and by excess of Cu. 2·64 per cent. In 1877, Mörner prepared a copper albuminate with 1·2 per cent. of Cu, and another with 1·85 per cent. In 1887, Chittenden and Whitehouse obtained copper albuminate containing from 0·71 to 1·21 per cent. of Cu, but found that by redissolving and reprecipitating the percentage could be raised to 2·19. Now with our formula for $CuSO_4$, and the fact that Mellanby's globulin solution contained 0·024 gramme of globulin per cubic centimetre, we get $0{\cdot}024\, p = 88\, c$, that is to say, that at the earlier stages of precipitation $CuSO_4$ throws down 88 times its own weight of globulin. Thus, neglecting the small amount of Cu left in solution, we can calculate that the copper globulinate of Mellanby's experiment contained 0·45 per cent. of Cu. If all the globulin is precipitated, our equations give that 0·55 of the precipitate contains 0·45 per cent. of Cu, and 0·45 of it contains 0·83 per cent. of Cu, so that the precipitate as a whole contains 0·62 per cent.

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* See F. N. Schulz, 'Die Grösse des Eiweissmoleküls,' Jena, 1903.

These are only about one-third of Mörner and Harnack's values for the albuminates. But the larger value, 0·62 per cent., is close to the smallest of Chittenden and Whitehouse's, namely, 0·71. In the same way, from our equations for Mellanby's $ZnSO_4$ data, we find that the first precipitate of zinc globulinate contains 0·61 per cent. of Zn, and that if all the globulin is thrown down the compounds average 1·22 per cent. of Zn. Now Chittenden and Whitehouse found 0·91 per cent. of Zn in their zinc albuminate. So there is a fair correspondence between the composition of Mellanby's globulinates and Chittenden and Whitehouse's albuminates of Cu and Zn. Thus it bears upon the present inquiry to look more closely into the albuminates of these investigators. The following table contains, in the first row, the fraction f of metal in unit mass of its albuminate, in the second the equivalent mass E of the metal, and in the third the ratio f/E.

Table IV.

	Cu.	Zn.	Fe.	U.	Pb.	Hg.	Ag.
$10^2 f$	71	91	95	460	250	289	409
E	32	32·5	28	120	104	100	108
$10^3 f/E$	22	28	34	38	24	29	38

From the mean value of the last row we find the percentage of heavy metal in its albuminate to be 0·03 E. Hence the minimum molecular mass for albumin in the albuminates is 100/0·03 or 3300 nearly. Hence, we infer from the above argument that Mellanby's data would agree with the altogether independent data for the albuminates in making the minimum possible molecular mass for globulin about 3000. This result will be used in Section 6.

We shall now discuss the precipitation by acids of globulin dissolved in neutral salts. This is a very characteristic point in the chemistry of globulin. While a little acid dissolves globulin, it precipitates it from solution in salts. One would expect *a priori* from the similarity of salts and acids that they would help one another in dissolving globulin. Instead of that they are antagonistic. Hardy has shown that the solvent power of two neutral salts for globulin is the sum of their separate powers. All the more striking, therefore, is the antagonism of neutral salts and acids. It seems to me that the explanation of this fact is to be found in this, that the acids enter into the globulin molecule in different places from the neutral salts, because the H atom can play a different *rôle* in organic compounds from that possible to metallic atoms. The ordinary organic compounds are far more stable than the organo-metallic.

Moreover, the molecule of acid and of neutral salt enter the globulin not only in different places, but in places which make them incompatible with one another, unless special provision is made to keep them in position. Each is stable in its own place. Together they tend to instability. Hence, acids and neutral salts inhibit one another's action in dissolving globulin. We can apply this idea quantitatively to Mellanby's experiments in which acid is made first to precipitate globulin from solution in salts, and then to redissolve the precipitate. He used his solutions in such a way that the total amount of globulin and of neutral salt in the 10 c.c. of mixture under observation remained constant in a set of experiments. The strength of the neutral salts was 0·005, or 0·003 gramme per cubic centimetre, and of the globulin sufficient to saturate the salts. The amount of acid present in any case in the 10 c.c. is specified by the number of cubic centimetres of N/100 acid from which it could be obtained. The results show that up to maximal precipitation the fraction of the globulin precipitated is practically proportional to the amount of acid present. In fact, Mellanby's graphs, in which p the fraction of globulin precipitated is ordinate and amount of acid is abscissa, could be replaced by two straight lines, one running up from the origin to the point of maximum precipitation, and the other running down again to a point of zero precipitation or complete solution.

To discuss the theory of the whole action, let us denote by a the cubic centimetres of acid which in the absence of salt would just dissolve all the globulin. Let b be the number producing maximal precipitation in a given salt solution containing the globulin. Then b would dissolve the fraction b/a of the globulin in the absence of salt. But the salt present is able just to dissolve all the globulin by itself, hence we may say that of the total solvent power of the salt and the acid b, the salt and acid possess the fractions $1/(1+b/a)$ and $(b/a)/(1+b/a)$ respectively. So if p_m is the maximum fraction of the globulin precipitated by b, then $1-p_m$ is the fraction held in solution by acid, and the fraction held in solution by the salt is $\frac{1-p_m}{1+b/a}$.

Hence, by the presence of the acid b the salt is deprived of $1-(1-p_m)/(1+b/a)$ of its solvent power, and this gives us a measure of the inhibition. The amount of acid required to inhibit all the salt will be $b\div\{1-(1-p_m)/(1+b/a)\}$. But to dissolve all the globulin as well as to inhibit the salt we shall need an additional a of acid. Let r be the total experimental amount of acid that will just give a clear solution again, then

$$\frac{b}{1-(1-p_m)/(1+b/a)}+a=r. \qquad (9)$$

This is an equation for calculating a from the observed values of p_m, b, and r. For a solution containing 0·005 gramme of NaCl per cubic centimetre, with HCl and H_2SO_4 as acids, Mellanby found $p_m = 0{\cdot}15$ and $b = 2{\cdot}5$, while $r = 7{\cdot}3$. Solving (9) for the positive root of a, we get $a = 2{\cdot}8$. From all his data the following table has been prepared for four salt solutions of the strength specified:—

Table V.

	$NaCl$ 0·5 per cent.	Na_2SO_4 0·5 per cent.	$MgSO_4$ 0·3 per cent.	$MgCl_2$ 0·3 per cent.
10^2p_m	15	9	17	7
$10b$	25	45	40	39
$10r$	73	90	100	67
$10a$	28	25	39	15

These strengths of solutions dissolve nearly equal amounts of globulin in 10 c.c., but not quite equal. If we calculate the relative amounts of globulin by the principles of Section 1, we find that they are as 1·7, 2·1, 2·0, and 1·9. Hence, relative values of a, referred to equal amounts of globulin, will be derived from the values of $10a$ in the table by dividing by these numbers, giving 17, 11·4, 17·5, and 9. Now these are to one another nearly as the values of 10^2p_m in the table, a fact which confirms in a general way the above reasoning and calculation, though, strictly, instead of comparing with p_m, we should compare with p_m referred to equal mass of globulin for each salt. In a broad way a tends to be proportional to p_m.

It is a striking fact that though the acids HCl and H_2SO_4 act by equivalents in each case, the same number of equivalents is not required in different cases; in other words, a for different solvents and a constant amount of globulin is not a constant, although with pure globulin these acids react in definite proportions. It seems, indeed, as though the precipitate we are now discussing cannot be pure globulin. So it is noteworthy that the amounts of acid required to yield p_m are nearly proportional to the cube of the total globulin strength in each of the above four cases, for, taking the values of $10b$ from Table V and dividing them by the cubes of the relative globulin strengths given above, we get $25/1{\cdot}7^3$, $45/2{\cdot}1^3$, $40/2^3$, and $35/1{\cdot}9^3$, or 5·1, 4·9, 5·0, and 5·1. It would appear, then, that the equation of chemical equilibrium between acid b and total globulin G, dissolved in neutral salt, is of the form

$$G^3 = kb. \tag{10}$$

Here, evidently, G^3 represents the mass action amongst three globulin compounds, whose amount in each case at the point of maximal precipitation is proportional to the total amount of globulin present. Of these three we know that two are probably the compound of globulin with acid alone and the compound with neutral salt alone. The third, then, is probably a compound containing both acid and salt, stable only when the other two are present in excess. These compounds form by their joint action the precipitate, whose rate of formation is measured by G^3. Then in (10) kb must represent the rate of action of the acid tending to dissolve the precipitate, which acts in the usual way with a constant active mass.

It is worth noting that when NaCl and $MgSO_4$ are the solvents, acids precipitate globulin nearly equally, while when Na_2SO_4 and $MgCl_2$ are the solvents, acids precipitate nearly equal fractions, but smaller than in the previous case. It seems as if ions of the same valency, like Na and Cl or Mg and SO_4, can co-operate better than dyad with monad as found in Na_2SO_4 and $MgCl_2$. This recalls the observation of Picton and Linder, that ions of different valency inhibit one another in precipitating colloids, while ions of the same valency co-operate.

4. *A Theory of the Colloidal State.*

The characteristic of the colloid state is that the molecules cease to have a separate existence; they link on to one another by means of the atomic electric charges, thus forming the meshes so characteristic of colloids

Each particle in a suspension of a globulin might, therefore, be called a molecule, but with no advantage. In each such particle, however, a certain pattern is repeated in three-dimensional space. In a colloid this pattern is the real subject of the chemist's study, just as the molecule is in crystalloids. Because of its importance, I propose to call this pattern a semplar. An imaginary case will enable the theory to be presented in a definite form. The nitrogen of NH_3 acts trivalently, but it can act also with pentad valency. In the old phraseology two of the bonds of N in NH_3 were said to satisfy one another. Now, in Helmholtz's theory, valency is the number of electrons associated with an atom. I have proposed to consider variable valency as due to the existence of opposite electrons in the atom showing it,* and for convenience of notation and printing I have proposed to denote the positive electron by $\sharp$ and the negative by $\flat$. Thus both the triad and pentad valencies of N are expressed by writing it $\sharp N\flat_4$, because $\sharp$ and $\flat$ can coalesce to form a doublet $\sharp\flat$, in which each electron almost completely neutralises the chemical activity of the other. On the same principle the symbol of the hydrogen ion is $H\sharp$, so that in NH_3 there are three doublets $\sharp\flat$ chemically effective and one ineffective. I have shown that these doublets and others are the cause of cohesion and rigidity. In a crystal of frozen NH_3 the molecules are all separate, each affecting its neighbours by the action of these doublets upon one another. But imagine each ineffective doublet of N to be made effective, so that $\sharp$ reaches out to unite with $\flat$ of a neighbour N, then every molecule of NH_3 becomes chemically linked with two of its neighbours, it becomes a semplar, and the whole crystal becomes a mesh of such semplars. Under certain conditions it could retain its crystalline form. This imaginary example explains the nature of a colloid on the present theory, and shows how the transition from crystalloid to colloid can be continuous with occurrence of crystalline colloids. The formation of polymers is due to ineffective doublets becoming effective, though generally the linking up of molecules is restricted to groups of m molecules, where m measures the amount of polymerisation. The most important case is that of water, which I have shown† to be a mixture of $(H_2O)_3$ trihydrol, found pure in ice, and of $(H_2O)_2$ dihydrol. In water and other polymers we have the simplest instances of semplars. When m, the measure of polymerisation, becomes large and indefinite, it gives us the colloid state, in which chemical forces between neighbour semplars produce effects of the same order as those between the separate doublets of molecules in the solid state. Before applying this theory to the chemistry of globulin, it will be helpful to discuss with its aid the remarkable connection, discovered by Schulze and investigated by Picton and Linder, between the valency of ions and their coagulating power. Hardy has shown that a similar relation holds for certain organic colloids. A theoretical explanation of it has been offered by Whetham,* based upon considerations as to the probability of electrolytic ions of like sign coming together. Though suggestive, this theory seems to me inadequate, because the electric repulsion between ions of like sign tends powerfully to keep them apart. On the present theory, coagulation consists in opening out ineffective doublets ♯♭ so that they link together neighbour molecules of the coagulating substance. Hence ionic charges must sometimes be instrumental in coagulation, because a free ♯ tending to attract ♭ in the doublet and to repel ♭ is an agent for opening out ineffective doublets. Suppose that a free ♯ unites with ♭ from an ineffective ♯♭ of a molecule of coagulee, then it liberates ♯, which will grasp the ♭ of a neighbouring ineffective ♯♭, and so the coagulation spreads until perhaps the other ion of the electrolyte with its electron ♭ steps in and arrests the process. But there is another way in which coagulation can be propagated from an ion as centre. The electric force of the ion will act on the ineffective doublets out to a considerable distance from itself, and, moreover, every ineffective doublet which is made chemically effective alters the local electric equilibrium by the change. Each coagulated molecule is a centre of coagulation. Hence we have the speed of coagulation due to an ion proportional to the electric charge of the ion and to the amount of coagulation already produced by it. Let c be the coagulum produced by electrolyte of concentration s, then we have, with ν for valence of the effective ion and k a constant,

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* 'Phil. Mag.,' [6], vol. 3, p. 161.

† 'Phil. Mag.,' [5], vol. 50, p. 460.

$$\frac{d}{dt}\left(\frac{c}{s}\right) = k\nu\frac{c}{s} \quad \therefore \quad \log c/c_0 = k\nu\,(t-t_0), \qquad (11)$$

where c_0 is the amount of coagulum produced at a certain short time t_0 after the beginning of the process. This small initial coagulation is produced mostly by the first process of direct linking, and is proportional to νs the total number of electrons which start the linking up, so we may write

$$\log c/\nu s = k\nu\,(t-t'_0). \qquad (12)$$

Now, in the experiments, the number of equivalents νs of electrolyte required to produce a certain amount of coagulation c in a very short time are measured. We may neglect t'_0 and take t as well as c to be the same for each electrolyte. If we call $1/\nu s$ the coagulating power of the electrolyte, equation (12) makes its logarithm linear in the valency ν. Schulze found the coagulative powers of chlorides of mono-, di-, and tri-valent ions on arsenious sulphide to be as 1:30:1650, the common logs of which are 0, 1·477 and 3·217, which fall nearly into the linear form 1·564 $(\nu - 1)$.

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* 'Phil. Mag.,' [5], vol. 48.

The data of Linder and Picton for the corresponding sulphates on arsenious sulphide are as 1:35:1023, whose logs are 0, 1·544, and 3·01, which may be written 1·515 $(\nu - 1)$.

The present theory, then, lends itself well to an explanation of this remarkable law.

In the chemistry of globulin, the prominent fact is that salt solutions dissolve from a suspension an amount nearly proportional to the original total strength of the suspension. According to the present theory, the explanation is as follows: Just as ions by their elective force draw out the ineffective doublets of molecules, which they cause to join up as semplars, they can, under suitable energy conditions, break the doublets joining the semplars, which they liberate as molecules. For example, if a crystal of our imaginary colloid NH_3 were dissolved in HCl, the action would be one in which the ions H and Cl would break the doublets joining the semplars NH_3, and would join on to form NH_4Cl. This I take to be a representation of the solution of globulin in acids, the action of salts being not so simple. It is a corollary of the coagulation of colloids by electrolytes. The same electric actions that build up a coagulum can sometimes pull it down, especially if the operating ions can step into places which make the total chemical potential energy smaller than before. Now the number of semplar doublets in a globulin suspension which the ions can break will either be the total thereof or a certain fraction of the total. In the former case we should have solution of the colloid in the ordinary way, as by acids. In the latter case the ions will only break those doublets which can be made unstable by the particular attacking force of ions used. Hence the fraction of a globulin particle dissolved by a given solution will have a definite value, and the amount of globulin dissolved will be proportional to the total number of particles, that is, to the original concentration of the suspension. This is the explanation of the most striking experimental result brought out by Hardy and Mellanby. We now proceed to give the corresponding interpretation of equation (1) deduced from Mellanby's experiments. Let us denote the semplar of globulin by G. The simplest possible formula for the semplar at the moment of its detachment is $\flat G \sharp$. Then either $\sharp$ and $\flat$ can coalesce to form an ineffective doublet, leaving G as a molecule, or if ions like Na and Cl are present, their electric charges will link them on, giving $Na\sharp\flat G\sharp\flat Cl$, or, more briefly, NaGCl. Now the fact that about 150 times as many equivalents of NaCl as of HCl are required to keep a given amount of globulin in

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solution, shows that NaGCl must be relatively very unstable; a large excess of Na and Cl ions is required to keep it in existence. Its existence depends on the breaking of doublets and keeping them broken. The number of bonds broken per second by the Na and Cl ions will be proportional to the concentration c of these ions and to the number of breakable bonds remaining to be broken, that is to $G-g$. The rate of solution can be written $k_1 c\,(G-g)$. Now the rate at which the dissolved g tends to go out of solution will be proportional to g and to a function of c, which just vanishes when $c = C$, the concentration which dissolves all the globulin. The simplest such function is $C-c$, so we write the rate of precipitation as $k_2 g\,(C-c)$. So, for equilibrium,

$$k_2 g\,(C-c) = k_1 c\,(G-g), \quad \text{therefore} \quad p\,(1-q) = Aq\,(1-p) = \frac{A}{C}\,c\,(1-p), \tag{13}$$

which is identical with (1) and (2). Now A is the ratio of k_1, the velocity of solution of the globulin, to k_2, the velocity of precipitation. If instead of c we use the number of gramme molecules c/M in these equations, its coefficient becomes MA/C, which gives a measure of the ratio of the velocities of solution and precipitation when gramme molecules of different salts are compared as to their actions on globulin of a given concentration. Now the velocity of solution of globulin by a molecule will be proportional to the rate at which the doublets ♯♭ are broken by ionic charges, so it will be proportional to the velocity of the ion and its charge, that is to say its valence. But in the precipitation of globulin the ions have to be extracted from combination with the globulin, so the number of doublets to be broken will be proportional to the valence of the ions. It is assumed here that in salt globulins an ion is loosely attached at the semplar doublet which it breaks, and that as breaking power is proportional to valence, so also is the strength of attachment. The velocity of precipitation will be therefore inversely as the valence. Hence MA/C will be proportional to the square of the valence, which is the modified form given to Mellanby's law in Section 1. As regards the dependence of the velocity of precipitation on velocities, it will arise mostly from motion of the combined ions in the globulin compound. We find, then, that MA/C must be proportional to the velocity of the ions and to a velocity depending on the globulin. Now the velocity of the ion must not be confused with the quantity defined in physics as the ionic velocity, which is a velocity generated under a certain unit external electric force. Apparently, in reacting with globulin, the different ions move with about the same speed. But as regards the effect of change of temperature on the velocity of ions, we know the velocity to be inversely as the viscosity of water at different temperatures. Hence, our equation (3) expresses both this dependence of MA/C on the viscosity of water and on velocity inside the globulin compound. Thus the present theory of colloids is able to give a consistent account of these important features of the chemistry of globulin. The precipitations of globulin hardly need review in this connection, as they have been pretty fully considered in Section 3.

5. *The Electric Conductivity of Globulin Solutions.*

Hardy, by overcoming the experimental difficulties of measuring these conductivities, has obtained data of great importance, which I propose to make more available by expressing them in interpretable formulæ. With HCl 1 gramme of globulin was dissolved in $9{\cdot}32 \times 10^{-5}$ gramme equivalents of acid, and the solutions prepared from this by successive dilution have their strengths given by v, the volume of solution containing 1 gramme equivalent of HCl. From the measured specific conductivity of these solutions the molecular conductivity of the HCl is calculated in the usual way, but it should be noticed that it is expressed with reference to a unit of resistance 10 times as large as that often used, so that conductivities are made 10 times as large. For HCl globulin solutions the molecular conductivity μ of the HCl given by Hardy[*] is expressed by—

$$1/\mu = 0{\cdot}00026 + 0{\cdot}009 v^{-\frac{1}{3}}, \qquad (14)$$

as the following comparison shows :—

Table VI.

v	107	217	434	868	1735	3470
μ calc.	465	568	690	830	991	1170
μ exp.	439	596	706	834	991	1167

At infinite dilution (14) gives $\mu = 3846$, the value for pure HCl being 3840. It appears, then, that at infinite dilution HCl globulin is entirely dissociated, yielding completely ionised HCl. The equation having so extensive a range must have fundamental theoretical significance, which is easily brought out. The occurrence of $v^{-\frac{1}{3}}$ reminds us of Kohlrausch's discovery that the molecular conductivity of dilute electrolytic solutions is a linear function of $v^{-\frac{1}{3}}$, a fact which I have sought to trace back to the 3 occurring in the formula $(H_2O)_3$ for trihydrol.[†]

For the molecular conductivity of an ion of radius a, valency ν, dielectric

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[*] *Loc. cit.*, p. 274.

[†] See "The Molecular Constitution of Water," and "Ionisation, Ionic Velocities, and Atomic Sizes," 'Phil. Mag.,' [5], vol. 50, and [6], vol. 3.

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capacity K, and charge e, moving through water of dielectric capacity K_0 and viscosity η the formula is—

$$\mu = ve^2 K_0/6\pi\eta a K; \tag{15}$$

that is to say, the ionic velocity is inversely as a, so that the time taken by an ion under standard electric force to travel 1 cm. is proportional to a. Now in the case of HCl globulin the corresponding time is proportional to $1/\mu$, and therefore to $0{\cdot}00026 + 0{\cdot}009v^{-\frac{1}{3}}$. Here 0·00026 represents the time that would be taken by the H and the Cl ions to increase their distance apart by 1 cm., if they were always dissociated from the globulin. The term $0{\cdot}009v^{-\frac{1}{3}}$ represents the time lost on account of the presence of the globulin. The way in which this time is lost is the following: Suppose that HCl globulin is HGCl, yielding two sets of ions $\mathrm{HG}\sharp$ and $\mathrm{Cl}\flat$, and also $\mathrm{GCl}\flat$ and $\mathrm{H}\sharp$. So the H ion travels part of its path as $\mathrm{H}\sharp$ and part as $\mathrm{HG}\sharp$, while the Cl ion travels part of its path as $\mathrm{Cl}\flat$ and part as $\mathrm{GCl}\flat$. Let τ be the fraction of each second which $\mathrm{H}\sharp$ and $\mathrm{Cl}\flat$ spend in the forms HG and GCl. During this fraction each moves with the smaller velocity u due to the large radius of G, for the rest of the second it moves with the ionic velocity u_0 appropriate to $\mathrm{H}\sharp$ or $\mathrm{Cl}\flat$. The average velocity of the ion is $u_0(1-\tau)+u\tau$. Hence the time taken by the H and Cl ions to separate by 1 cm. is proportional to—

$$\frac{1}{u_0(1-\tau)+u\tau} = \frac{1}{u_0}\left(1+\frac{\tau(1-u/u_0)}{1-\tau(1-u/u_0)}\right). \tag{16}$$

If, then, we write $0{\cdot}00026+0{\cdot}009v^{-\frac{1}{3}}$ in the form $0{\cdot}00026\ (1+34{\cdot}6v^{-\frac{1}{3}})$, we have

$$\frac{\tau(1-u/u_0)}{1-\tau(1-u/u_0)} = 34{\cdot}6v^{-\frac{1}{3}}. \tag{17}$$

Denote $\tau(1-u/u_0)$ by x, then we have $x/(1-x)$ proportional to $v^{-\frac{1}{3}}$, and therefore to $c^{\frac{1}{3}}$, where c is the concentration of the HCl. If u/u_0 is small, as Hardy directly proved it to be in the way we shall discuss in the next section, then x stands almost for τ, the fraction of the total H or Cl existing at any moment as $\mathrm{HG}\sharp$ or $\mathrm{GCl}\flat$, while $1-\tau$ stands for the amount of free HCl. In short, τ represents the fraction of the HCl which is combined, and $1-\tau$ that which is free. Hence the proportionality proved above, which may be written

$$x = k(1-x)c^{\frac{1}{3}}, \tag{18}$$

is an equation of chemical equilibrium expressing that the amount of combined HCl existing as HGCl in the form of ions HG and GCl is proportional to the amount of free HCl and to the amount of something else. In "Ionisation, etc."* it is suggested that this something else in ordinary electrolysis is $\sharp H_2O\flat$ broken out of trihydrol. It is shown how the number of these in a solution is proportional to $c^{-\frac{2}{3}}$, so that when they act upon the whole solute c they produce an agent of amount proportional to $c^{-\frac{2}{3}}c$, that is $c^{\frac{1}{3}}$, which, in the present case, acting on the residue $(1-x)$ of free HCl keeps the amount x of combined HCl formed. Hence, for equilibrium, we have $x = k\,(1-x)\,c^{\frac{1}{3}}$ as in (17) and (18). If in (16) we neglect u/u_0 and put $v = 1000$, we find $\tau = 0{\cdot}78$, that is to say, that N/1000 HCl, which is just saturated with globulin, contains 78 per cent. of the H and Cl ions in combination with globulin, and only 22 per cent. free. The experiments of Hardy, when summarised in (14), give a pretty complete insight into the chemistry of HCl globulin.

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* *Loc. cit.*, p. 173.

As regards alkali globulins, the data of Hardy can be expressed by

(1 gramme glob. diss. in $9{\cdot}7 \times 10^{-5}$ gramme equiv. NaOH)

$$\mu = 220 + 1/(0{\cdot}0005 + 0{\cdot}031v^{-\frac{1}{3}}),$$

(„ „ 18×10^{-5} gramme equiv. NaOH)

$$\mu = 290 + 1/(0{\cdot}0005 + 0{\cdot}031v^{-\frac{1}{3}}),$$

(glob. diss. in NH_4OH) .... $\mu = 290 + 1/(0{\cdot}00048 + 0{\cdot}023v^{-\frac{1}{3}})$.

(19)

We shall at first discuss only the first and third of these as characteristic of NaOH and NH_4OH. When v is infinite, the first gives $\mu = 2220$, while in the same units the standard data of Kohlrausch would give 444 for Na and 1740 for OH, that is $\mu = 2184$. The formula gives complete dissociation of the alkali globulin at infinite dilution. It is noteworthy that the first term in (18) for NaOH globulin is 220, which is half of the standard value for Na. It appears, then, that half the Na unites with globulin to form a globulinate, which dissociates completely at all the dilutions of Hardy's experiments, giving Na ions, and globulinic acid ions whose velocity is too small to make itself apparent. As the OH of this half of the original NaOH does not contribute to the molecular conductivity in the same way as the Na, but yet appears in the conductivity at infinite dilution, we see that it joins on to the globulin in a similar way to that of the other half of the NaOH. The H of the globulin displaced by the Na atom must also join on to the globulin in a different function. The other half of the NaOH seems to combine with globulin in a way similar to that of HCl. A compound such as NaGOH seems to be formed (with H and OH attached from the first half of the NaOH), yielding ions $NaG\sharp$ and $GOH\flat$ which may be practically two varieties of the globulinic acid with which the other half of the Na combines. From the second of equations (19) it appears that if twice as much NaOH is used as suffices to dissolve all the globulin, its molecular conductivity is but little changed. So in this case a quarter of the base reacts in quite a different way from the remaining three-quarters. The same considerations apply to the case of NH_4OH. The first term 290 is close to the 311 which the standard datum of Kohlrausch would give if half the NH^4 existed as basic ions taking the place of the H ions of globulinic acid. The other half of the NH^4OH combines additively like the second half of the NaOH. In the alkali globulins we must distinguish between the basic half of the alkali and the additive. To the additive half exactly the same considerations apply as to the additive HCl globulinate, and the degree of dissociation of the additive alkali hydrate can be determined as in the case of HCl globulin. Hardy found that to neutralise globulin to phenolphthalein with NaOH the amount of alkali required is twice that needed to dissolve the globulin. Here we have additional evidence that the alkalies act in two ways. Hardy finds that 1 gramme of globulin is dissolved by 10^{-4} gramme equivalents of alkali, and by 2×10^{-4} of acid. Now only half the alkali displaces acidic H, hence the acidic H atoms are only one-quarter the number of places where HCl can add itself to the globulin molecule. Mellanby's measurements on horse globulin do not agree with Hardy's distinction between the number of equivalents of acid and alkali needed to dissolve globulin. He finds a gramme of globulin dissolved by about 10^{-4} gramme equivalents of HCl and about the same number of NaOH. Globulins of different origin and history appear to differ to an extent that limits us at present to a discussion only of orders of magnitude in this connection.

Concerning the molecular conductivity of neutral salt solutions saturated with globulin, Hardy made some measurements. NaCl of concentration 0·16 N had its conductivity depressed 2·4 per cent., of 0·11 N by 2·3 per cent., and of 0·09 N by 2·1 per cent. While of $MgSO_4$ 0·3 N the lowering of conductivity was only 1·4 per cent.

Hardy does not give the strength of the globulin suspension with which he saturated his salt solutions, but states that a gramme of globulin was dissolved by from 10^{-2} gramme equivalents up to 2×10^{-2}. About 100 times as many equivalents of NaCl are required to keep globulin in solution as of NaOH, but as the globulin reduces the conductivity of NaCl about 2 per cent., we may take 2 per cent. of the salt to be combined with globulin, so two equivalents of NaCl actually unite with globulin for one of NaOH. Thus it appears that NaCl in its action on globulin is liker to HCl than to NaOH. The conductivity measurements fully confirm the conclusions arrived at from the solvent powers of neutral salts, namely, that the compounds formed are very unstable and require a large excess of salt to maintain their existence.

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6. *The Molecular Mass of Globulin.*

In "A Dynamical Theory of Diffusion for Non-Electrolytes and the Molecular Mass of Albumin,"[*] I have sought to show how the molecular radius of a molecule can be found from its coefficient of diffusion, having previously obtained the corresponding relation reproduced as (15) in the present paper between molecular conductivity and radius. These two definite and accurately measurable velocities, the ionic and that of diffusion, are convenient aids in the measurement of large molecular masses. From the Graham-Stefan coefficient of diffusion for egg albumin the radius of a gramme molecule of albumin was found to be 30 cm., whence, from the percentage composition, the following formula is obtained for albumin, $C_{1436}H_{2364}N_{359}O_{482}S_{15}$ with a molecular mass 32,814. Now Hardy has obtained a direct measurement of the ionic velocity of globulin, namely, about 10^{-4} cm. per sec., which corresponds to a molecular or rather ionic conductivity 100 in the units used by Hardy, and 10 in the units of Kohlrausch. With the latter units (15) is transformed[†] to

$$B^{\frac{1}{3}} = 280\nu/\mu K, \tag{20}$$

in which K is the dielectric capacity of the ion, having for globulin the value 2 nearly, because for proteids the index of refraction which equals $K^{\frac{1}{2}}$ is about 1·4. B is the volume of the molecules in a gramme molecule of the ion, and ν its valence. We are going to use this equation to find B for globulin, using Hardy's value 10 for μ. But so far ν is unknown. It is the basicity of globulinic acid which is easily determined approximately by the facts we have in hand. Thus it has been shown that when NaOH dissolves globulin, half of it acts as a relatively strong base. Let M be the molecular mass of globulin, then ν gramme molecules of NaOH act as a strong base with M grammes of globulin. But Hardy finds 10^{-4} gramme molecule of NaOH just dissolves 1 gramme of globulin, so $10^{-4}/2$ gramme molecule satisfies the ν-basicity of globulin

$$2 \times 10^4\nu = M. \tag{21}$$

With $\mu = 10$ equation (20) gives

$$B^{\frac{1}{3}} = 14\nu. \tag{22}$$

For the percentage composition of globulin, we have Hammarsten's determinations, with which I shall give the limiting volume of a gramme atom of the elements taken from my 'Phil. Mag.' papers on molecular force.

	C.	H.	N.	O.	S.
Per cent.........	52·71	7·01	15·85	23·32	1·11
Atomic B	8	4	8	6	18

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[*] 'Phil. Mag.,' [6], vol. 9, 1905.

[†] See "The Dielectric Capacity of Atoms," 'Phil. Mag.,' [6], vol. 7.

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Let x be the number of atoms of C in the molecule of globulin, then

$$12x = 0{\cdot}5271\mathrm{M}, \tag{23}$$

and also

$$x\left\{8+\frac{12}{52{\cdot}71}\left(4\,.\,\frac{7{\cdot}01}{1}+8\,.\,\frac{15{\cdot}85}{14}+6\,.\,\frac{23{\cdot}32}{16}+18\,.\,\frac{1{\cdot}11}{32}\right)\right\} = \mathrm{B}. \tag{24}$$

These four equations (21, 22, 23, 24) give us the four unknowns ν, x, B, and M. The best way of solving them is to give ν integral values, and find which value makes the four equations as consistent as possible within permissible limits of error. With $\nu = 1$ (21) gives M = 20,000, and then (22) gives B = 2744, which in (24) yields $x = 147{\cdot}7$, and this in (23) makes M = 3362. As they stand, the two values of M, namely 20,000 and 3362, are inconsistent. If $\nu = 2$, the first becomes 40,000 and the second 8×3362 or 26,896. For $\nu = 3$ the comparison is between 60,000 and 3362×27 or 90,774. No value of ν above 3 is permissible. Hence the choice for the formula of globulin is between one which makes it a dibasic acid of molecular mass about 40,000 and another which makes it a tribasic acid of molecular mass about 60,000. Let us inquire what changes in the experimental data would make the four equations strictly consistent for $\nu = 2$ and $\nu = 3$. The most uncertain number is Hardy's valuable datum $\mu = 10$. For a transparent opalescent dialysed solution of NaOH globulin he found $\mu = 7{\cdot}66$ and estimated 10 as the probable limit to which the value would rise with complete ionisation of the compound. But his solution being opalescent, there must have been some electric endosmose such as he found with H$\bar{\mathrm{A}}$ globulin, for which it raised μ up to 23. In the observed 7·66 for NaOH globulin there is already some compensation of errors, and it is an open question whether the limit is more or less than 10. It is easy to calculate the value of μ in (20) which would make $\nu = 2$ and M = 40,000 satisfy (21, 23, 24). It is 8·76. So for $\nu = 3$ and M = 60,000 we should need $\mu = 11{\cdot}50$. Hence the decision between the two alternatives should be reached by a sharper determination of the ionic velocity of globulin or by its coefficient of diffusion, or preferably by both of these methods. Probably 8·76 is the better value for μ. With this and $\nu = 2$ and M = 40,000 we get $x = 1756$. Hence the formula of globulin is $C_{1756}H_{2804}N_{452}O_{584}S_{14}$.

This formula of the globulin molecule furnishes a molecular mass 1·22 times that given above for egg albumin obtained from the Graham-Stefan coefficient of diffusion. That formula can be greatly simplified for discussion if we replace the number of carbon atoms 1436 by the round number 1440, the 2364 of H by 2400, the 359 of N by 360, and the 482 of O by 480, and neglect for a while the 15 for S. These changes keep the percentage composition of egg albumin true within the limits of analytical error, and enable us to write the main part of the egg albumin molecule as 120 ($C_{12}H_{20}N_3O_4$).

The formula of globulin cannot be quite so much simplified, but we can write it $146(C_{12}H_{19}N_3O_4)+14N$, neglecting S meanwhile. The group $C_{12}H_{20}N_3O_4$, which is repeated 120 times in the albumin molecule, is closely related on the one hand to the polypeptides synthesised by E. Fischer, and to the pure peptones prepared by Siegfried. For the derivation of dipeptides and tripeptides from the amino acids by abstraction of water we have the equations*

$$2NH_2(CH_2)_nCOOH-H_2O = NH_2(CH_2)_{2n}NHCOCOOH, \text{ dipeptide};$$
$$3NH_2(CH_2)_nCOOH-2H_2O = NH_2(CH_2)_{3n}(NHCO)_2COOH, \text{ tripeptide.}$$

If, now, in this general formula for a tripeptide we put $n = 3$, we obtain $C_{12}H_{23}N_3O_4$, a group containing 3H more than our typical albumin group above. If, then, we remove 3H from this polypeptide, we obtain a trivalent radicle, $C_{12}H_{20}N_3O_4$, which we find repeated 120 times in the molecule of egg albumin. It hardly matters at present how many times CH_2 may be removed from one of these groups and inserted into others, for we are considering only the broad plan on which the albumin molecule is built.

Similarly, the properties of globulin depend on a certain preponderating group whose average composition is close to $C_{12}H_{19}N_3O_4$. These groups are almost as closely related to the pure natural peptones prepared by Siegfried as to the synthesised polypeptides of E. Fischer.† These are the antipeptones α and β, or trypsin-fibrin peptones $C_{10}H_{17}N_3O_5$ and $C_{11}H_{19}N_3O_5$, of which the next homologue would be $C_{12}H_{21}N_3O_5$, differing from our albumin group by OH and from our globulin group by OH_2. Even if these formulæ need to be doubled, the relation would not be altered. Then come the pepsin-fibrin peptones α and β, $C_{21}H_{34}N_6O_9$, $C_{21}H_{36}N_6O_{10}$. It is significant that the latter could be produced by the additive union of the antipeptones α and β.

Now, the experiments of W. Neumann and Siegfried have yielded the result that in the antipeptones the H ions are to the OH ions as 2 : 1, and in the pepsin peptones as 3 : 2. But the number of H ions gives the number of COOH radicles in the molecule, while that of the OH ions gives the number of NH_2 radicles. Hence we infer that the group $C_{12}H_{19}N_3O_4$, when converted into an antipeptone, contains COOH twice and NH_2 once. Hence, in the joining up of such groups to the number of 146 to form globulin, there must be a large amount of internal salt formation, since COOH occurs only two or three times in the complete globulin molecule. It is most probable that the internal salt formation goes on in such a way that COOH and NH_2 neutralise one another.* Thus in the extreme case of all the oxygen existing as COO in $C_{12}H_{19}N_3O_4$, two-thirds of the total nitrogen would be present as NH_2 joined up with COOH in the globulin molecule. When the globulin molecule is made an ion, it has probably only two COO radicles showing the acid character. Hence we infer from our discussion of Hardy's conductivity measurements that the globulin molecule contains NH_2 eight times in such a form that HCl can join on as it does with NH_3 to form NH_4Cl. These eight NH_2 and the two COOH are the only ones left free out of about 290 of each sort existing in the globulin molecule. By more or less drastic treatment the latent NH_2 and COOH radicles may be called into partial action, and so globulin and albumin may be made to show apparently higher acidity and basicity than the eight and the two determined above. In the same way arise the discrepant results of different experimenters, discussed in Section 3, as to the percentage of heavy metals in their compounds with albumins. Different treatments open out or break up the albumin molecule differently. But yet a rather striking agreement appears in these discordant results if we regard them in the following way. The range in the percentages of Cu in albuminates and globulinates is 0·45 in Mellanby's globulinate, 0·71 in the albuminate of Chittenden and Whitehouse, 1·2 Mörner, 1·35 Harnack, 1·85 Mörner, 2·19 Chittenden and Whitehouse, 2·64 Harnack, and 11, the average of Ritthausen's measurements with vegetable albumins. For the weight of proteid combined with a gramme equivalent of Cu (31·6) these give the numbers—

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* See Cohnheim's 'Chemie der Eiweisskörper,' p. 59.

† Siegfried, 'Ber. d. Deutsch. chem. Ges.,' vol. 33; 'Zeitsch. f. Physiol. Chem.,' vols. 35 38, 45.

7020	4450	2630	2340	1710	1440	1200	287
24·1	15·3	9·05	8·04	5·87	4·96	4·10	0·987

which are expressed in the second row as multiples of 291, these multiples being all nearly exact integers 1, 4, 5, 6, 8, 9, 15, and 24. Here, then, we have evidence of the splitting up or opening out of the albumin molecule into parts, each of which contains an integral number of a group whose molecular mass is about 291, which is quite close to the 270 of our $C_{12}H_{20}N_3O_4$. This group with H_2O would give 288. This seems a fairly conclusive proof that there is a large amount of internal salt formation in albumins. The 3300 found in Section 3 for the mean mass of albumin combined with an equivalent of heavy metal in Chittenden and Whitehouse's experiments represents about 12 of our groups. It is interesting to note that the treatment of a vegetable albumin with copper breaks up the albumin molecule into parts of about the size of a peptone molecule. This probably accounts for the powerful toxicity of copper towards those lower plants to whose protoplasm it gains ready access.

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* See Mann, 'Chemistry of the Proteids,' pp. 210 and 211.

The large molecular mass of about 40,000 found for globulin accounts for the fact emphasised by Hardy that the polybasic acids H_2SO_4, H_3PO_4, combine by molecules and not by equivalents with globulin. The eight NH_2 radicles are so widely distributed over the large molecule that only one can come within the reach of an acid molecule. Thus only one OH radicle in H_2SO_4 and H_3PO_4 can operate. In the same way, since globulin contains only two COOH radicles, these are probably far apart, and a divalent atom like Ba can replace only one H. It would be possible for Ba to unite two globulin molecules through a COO radicle in each, and for SO_4 to unite two through an NH_2 radicle in each. Probably the colloidal state of a globulin suspension is due to the ordinary ineffective doublet of trivalent nitrogen becoming effective, thus enabling the globulin molecules to grip each other by means of the eight free NH_2 radicles in each. Solution in acid occurs when the acid satisfies the extra valencies of the NH_2 radicles, thus breaking their hold on one another. The semplar G of globulin escapes into the freedom of solution as part of the molecule $G(HCl)_8$. In edestin, T. B. Osborne has found what he calls the monohydrochloride insoluble, while the dihydrochloride is soluble. The explanation of this is that in the monohydrochloride sufficient junctions amongst the semplars are still left intact to keep the colloidal monohydrochloride from going into solution. The equivalent weights, 14,300 to 7000 given by Osborne for edestin, are 1/3 and 1/6 of the 40,000 found above for globulin.

As to solution of colloidal globulin in bases, we have seen that in the case of Hardy's ox globulin it was not sufficient to supply merely enough NaOH to form the easily dissociable globulin salt, but twice as much was required for complete solution. Here the extra NaOH is required along with OH and H due to the first half of the NaOH to break the colloidal junctions between the semplars. How it does this as efficiently as four times the equivalent HCl forms an interesting chemical question. The probable mode of operation of neutral salts in dissolving colloidal globulin has already been discussed.

Summary.

By expressing the experimental results of Hardy and Mellanby in simple formulæ, it is shown that the solution of globulin and its precipitation take place under simple conditions of chemical equilibrium. For example, if p is the fraction of a globulin suspension dissolved in a salt solution whose concentration is the fraction q of C that is required just to dissolve the whole

of the suspension, we get equation (1) $p(1-q) = Aq(1-p)$, in which A is the ratio of a velocity of solution to a velocity of precipitation. Mellanby's discovery of the dependence of M/C on valency and ionic velocity is applied to MA/C, M being molecular mass, and it is shown that when temperature varies, not only does MA/C depend upon the viscosity of the solvent water, but also on a function of temperature given in equation (3) which expresses the part played by globulin. It is noteworthy that this function has a minimum value about 40° C., near the temperature of warm-blooded animals.

For the precipitation of globulin by excess of $(NH_4)_2SO_4$, equation (5) is established, namely $p(1+p) = 28{\cdot}8(c - 0{\cdot}152)$, p being the fraction which the precipitated globulin is of the whole and c the concentration of the $(NH_4)_2SO_4$ solution in grammes per cubic centimetre.

Then follow formulæ for the remarkable precipitation of globulin by acids from solution in neutral salts. From these it appears that three compounds of globulin react in producing this precipitate.

Section 4 is devoted to a theory of the colloidal state, namely, that a colloid consists of molecules which are chemically united neighbour to neighbour by the action of valencies which are usually latent. Cases of multiple valency like that of nitrogen and oxygen are best accounted for by the electron theory of valence due to Helmholtz, with the assumption that a single atom can contain both negative and positive electrons. Thus the nitrogen atom has four negative electrons and one positive. In trivalent nitrogen the positive electron neutralises one of the negative, so that the atom has only three effective negative electrons along with an ineffective doublet. But if this doublet is opened out, it can link on its atom to two neighbour atoms, the negative electron grasping a positive, and the positive a negative.

According to this chemical theory of the colloidal state, the term molecule ceases to have a useful meaning when applied to a colloid, so the term semplar is used to name that structure which is repeated like a pattern in three dimensions through a colloid. By suppression of the colloid-producing valencies of doublets, a mass of semplars is caused to fall into a collection of separate molecules. In illustration of the usefulness of this theory, it is applied to show the dependence of the coagulating power of an ion on its valence. It is then applied also to explain the remarkable fact that the amount of globulin dissolved by a given salt solution from a globulin suspension depends on the concentration of the suspension. The action of the ions of a neutral salt in dissolving globulin is treated as only another manifestation of the same electrical effect which enables them to coagulate arsenious sulphide. This theory of the colloidal state leads to a theory of equation (1) and of the laws of A in it, especially that MA/C is proportional to the sum of the squares of the valencies of the ions of the electrolyte.

In Section 5 the experiments of Hardy on the conductivities of globulin solutions are expressed by formulæ which admit of very simple interpretation, and connect these conductivities with those of ordinary electrolytes. At infinite dilution the HCl compound of globulin is completely dissociated, its conductivity being that due to the completely ionised HCl, the part due to the globulin being then too small to appear. For any other dilution the amounts of combined and free HCl can be calculated by equations (17) and (18). In the compounds of NaOH and NH_4OH with globulin the conductivities show that half of the base combines with the globulin in a way which proves it to be an acid, the resulting salt being completely dissociated and showing the conductivity due to Na or NH_4 alone, that of the globulinic acid being too small to appear. The other half of the base which is required to dissolve globulin completely combines with it in a way similar to that of HCl, namely, by addition.

In Section 6 globulin is shown to have probably a molecular mass 40,000 and a basicity 2, the alternative being a mass 60,000 and basicity 3. Further experiments like those of Hardy on the ionic velocity of globulin and also on the coefficient of diffusion of globulin solutions would decide the matter, though doubtless various globulins differ in mass and basicity. A group, $C_{12}H_{20}N_3O_4$, related to polypeptides and peptones is shown to be the predominant structure in albumins. The discrepant results of different experimenters on the precipitation of albumin by heavy metals fall into harmony when it is proved that they precipitated different integral numbers of a group such as this in combination with an equivalent of heavy metal.